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ATROPINE, DIAZEPAM, AND PHYSOSTIGMINE: THERMOREGULATORY EFFECTS IN THE HEAT-STRESSED RAT

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SUMMARY

We have previously reported that administration of atropine (A) to unrestrained, sedentary, heat-stressed rats resulted in a dose-dependent increase in heating rate (rate of rise of core temperature, °C/min). Additionally, we have demonstrated that the decrements in treadmill endurance and increments in heating rate of physostigmine (PH)-treated running rats can both be restored to control levels by pretreating the animals with A and diazepam (D). Our objective in the present work was to determine if the administration of D+PH to A-treated unrestrained, sedentary, heat-stressed rats (N= 16/group, 510-530 g) could improve their thermal tolerance. The following drugs were administered singly (at 10 min intervals) via lateral tail vein: vehicle-control (C), A (200 ug/kg), D (500 ug/kg), and PH (200 ug/kg). After drug administration, the rats were heat-stressed (Tamb= 41.5°C) until a core temperature of 42.6°C was attained when they were removed to a 26°C chamber. The heating rates (°C/min) and tolerance times (min) of the respective groups were: C- 0.02, 235; A- 0.08, 58; A+D- 0.06, 94; and A+D+PH- 0.04, 143. Administration of D with A significantly decreased heating rate, and D+PH more than doubled the thermal tolerance of A-treated rats. Thus, the combination of A+D+PH not only restores PH-induced performance and thermoregulatory decrements of rats exercised in a moderate environment, but also reduces A-induced heat intolerance.

For several years we have been interested in the effects of anticholinergic and anticholinesterase drugs on physical, physiological, and thermoregulatory responses to heat and exercise. Atropine, the prototype of anticholinergic drugs, inhibits evaporative cooling in man by suppressing sweat production (1,2,3) and in rats by reducing saliva secretion which is behaviorally spread for evaporative cooling (4,5). Both sweat and saliva secretions are cholinergically regulated (3), and Clubley et al. (6) have demonstrated that both are analogously inhibited by atropine in man.

We have used the sedentary heat-stressed rat model to determine the dose-response effects of atropine, and reported that the rate of rise of core temperature (heating rate) of the rat was the most sensitive index of anticholinergic activity (7). Additionally, we measured the heating rates of other anticholinergic drugs to determine a potency for these drugs relative to atropine, and we likewise quantitated the anticholinesterase potency of various carbamates to reverse the atropine-induced increase in heating rate (8).

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The effects of anticholinergic and anticholinesterase drugs on thermoregulation in the heat are primarily due to alterations in muscarinic cholinergic effect on salivation and sweating. However, anticholinesterase and to a lesser extent anticholinergic drugs are also active at neuromuscular junctions (3) with potentially important effects on physical performance. A more comprehensive evaluation of these nicotinic effects is possible when an exercise regimen is utilized following drug administration. Administration of the anticholinesterase physostigmine (PH) to rats resulted in reduced endurance and an increased heating rate; both decrements were restored to control levels by pretreating the animals with both the anticholinergic atropine (A) and the anticonvulsant diazepam (D) (9).

We have developed two experimental models: one (sedentary heat exposure) primarily tests thermoregulatory function and the other (treadmill exercise in a moderate environment) physical endurance capacity. Physostigmine administration, primarily because of its nicotinic effects, resulted in a decremented endurance which was alleviated by the admjnistration of A+D (9). Atropine administration, by virtue of its inhibitory effects on saliva secretion, elicited an increased heating rate during heat exposure (7,8). Consequently, we administered D and PH to atropinized rats (heat-stressed, unrestrained) to determine if this combination (D+PH) would restore full thermoregulatory ability to atropinized rats.

METHODS

Eight groups of 16 adult male Sprague-Dawley rats (Charles River, CD strain, 510-530 g) were used one time only. The animals were housed individually in wire-bottomed cages and maintained in an environmental chamber at 26°C and 50% rh. Lighting was controlled automatically (on, 0600-1800 h), and Purina rat chow and water were available ad lib except during experimental intervals.

TABLE I

Drugs, Doses, and Order of Administration

DRUGS

C vehicle control - 0.2 ml saline + 0.5 ml serum +0.2 ml saline
A atropine - 200 ug/kg in 0.2 ml saline
D diazepam - 500 ug/kg in 0.5 ml fresh rat serum
PH physostigmine salicylate - 200 ug/kg in 0.2 ml saline

INJECTIONS*

GROUP	1st	2nd	3rd
C	SALINE	SERUM	SALINE
A	A	SERUM	SALINE
D	SALINE	D	SALINE
A+D	A	D	SALINE
PH	SALINE	SERUM	PH
A+PH	A	SERUM	PH
D+PH	SALINE	D	PH
A+D+PH	A	D	PH

* 10 min apart, via lateral tail vein

Prior to the heat stress, each rat received 3 separate injections at 10 min intervals via a lateral tail vein. The drugs, doses, and order of administration for each of the 8 groups are presented in Table 1. Atropine (A, 200 ug/kg, as the sulfate, Sigma Chemical Co.) was dissolved in 0.2 ml sterile 0.9% saline while diazepam (D, 500 ug/kg, Valium^{R}, injectable, 10 mg/2ml, Hoffman-LaRoche Inc.) was diluted to 0.5 ml with fresh rat serum; physostigmine salicylate (PH, 200 ug/kg, Antilirium, Forest Pharmaceuticals) was diluted to 0.2 ml with saline. Serum was used to dilute the diazepam, because its own vehicle is caustic to vascular tissue, the drug is less soluable in saline, and the use of plasma or whole blood would have necessitated the use of an anticoagulant. Extensive preliminary research indicated that this dose of PH consistently elicited a 40% inhibition of plasma cholinesterase, measured spectrophotometrically (prepared test kit, Sigma Chemical Co., St. Louis, MO); however, no determination of cholinesterase activity was done in this study. This order of administration (Table I) was chosen because A was expected to have the longest duration of action and PH the shortest. Each dose is within the human clinical range for the respective drug when the formula of Freireich et al (10) is applied (comparable rat dose = 7x human dose on a per kg basis).

TABLE II

HEAT EXPOSURE AND EVAPORATIVE LOSS DATA FOR SEDENTARY HEAT-STRESSED RATS

GROUP (N=16)	EXPOSURE (min)	HEAT RATE (°C/min)	% WT LOSS (%)	S-S[1] (0-3)	S-TIME[2] (min)
C	235* ± 12[3]	.019* ± .001	8.0* ± 0.3	2.8 ± 0.1	41 ± 2
D	256* ± 15	.019* ± .002	8.7* ± 0.5	2.8 ± 0.1	43 ± 3
PH	257* ± 12	.018* ± .001	8.0* ± 0.3	3.0 ± 0	25* ± 3
D+PH	257* ± 11	.019* ± .002	8.5* ± 0.3	2.9 ± 0.1	27* ± 4
A+D+PH	143**+ 9	.038**+ .002	4.7**+ 0.3	2.4 ± 0.1	52 ± 4
A+PH	136**+ 14	.040**+ .004	4.3**+ 0.6	2.0 ± 0.2	42 ± 6
A+D	94* ± 13	.060**+ .004	2.8* ± 0.4	1.4 ± 0.2	55 ± 3
A	58* ± 3	.079* ± .003	1.9* ± 0.1	0.6 ± 0.1	-----

1 Maximum extent of saliva spread by the end of heat-stress (7). No significance testing was done because of the subjective nature of the measure.
2 Mean exposure time by which rats achieved at least a 1 for extent of saliva spread. Many A rats had not achieved a score of 1 by the end of heat exposure.
3 Values are mean + S.E.
* Significantly different ($p<.05$) from C.
+ Significantly different ($p<.05$) from A.

Fifteen min after the 3rd injection, the rats were heat-stressed unrestrained (so that they could actively spread saliva) in their own cages in a chamber (1 x 2 x 2 m) maintained at 41.5°C (electrically heated circulated air) and 30% relative humidity until a core temperature (Tco, flexible probe

inserted 6.5 cm) of 42.6°C was attained. At this time the animals were quickly removed from the heat, weighed and allowed to cool passively in the original 26°C chamber. The heat exposure does not appear to distress the animals, because they usually sit quietly or groom saliva into their fur during the heat exposure. During heat exposure, weight loss corrected for fecal pellet production, Tco, and the extent of saliva spread were monitored at 5-15 min intervals. The extent of saliva spread was graded on a scale of 0 to 3 as previously reported (7).

Statistical significance ($p<.05$) was determined by a one-way analysis of variance (11) followed by the Student-Newman-Keuls multiple range test for all pair comparisons (12).

RESULTS

In Table II, exposure time to a Tco of 42.6°C as well as heat rate, % wt loss, and extent of saliva spread (S-S) during the exposure time are presented for the non-atropinized groups in the top half and the atropinized rats in the bottom half. Except for the S-time column (time by which rats had achieved at least a 1 for extent of saliva spread), there were no significant differences among the 4 groups that did not receive atropine; however, all the values in the top half of Table II are significantly different from those of the 4 atropinized groups.

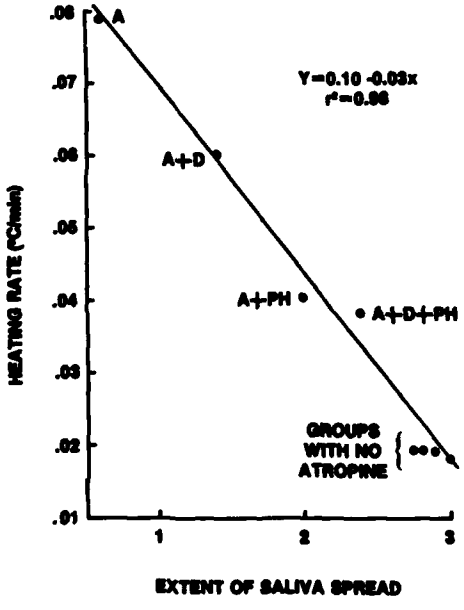

FIG. 1
Heating rate in °C/min is regressed against the extent of saliva spread (0= no spread, 3= ventral surface completely covered) by the end of the heat stress for all 8 drug groups (see Table I for identification of groups).

The groups receiving A in Table II are arranged in order of decreasing exposure time. As you move down Table II from A+D+PH to A, exposure time, % wt

loss, and extent of saliva spread all decrease and heating rate increases consistently. Note that the administration of either or both of D and PH with A significantly decreased the heating rate seen in the group with A alone, and PH or D+PH significantly increased exposure time to a Tco of 42.6°C and % wt loss. Data in Table II for % wt loss and extent of saliva spread are maximum values attained by the end of the heat exposure; these values might well be expected to increase with increasing exposure time. However, the values for % wt loss and extent of saliva spread at 60 min (the nearest measurement interval to the mean exposure time of the A group) were as follows: A+D+PH- 2.2%, 1.5; A+PH- 2.1%, 1.5; and A+D- 2.0%, 0.9. These data indicate that the patterns of final % wt loss and extent of saliva spread for the atropinized groups were well established even by the end of 60 min of heat exposure. PH stimulated earlier salivary secretion and spreading in the PH and D+PH groups, but this did not result in an increased exposure time or decreased heating rate in either of these groups.

Regressing the extent of saliva spread against the heating rate for all 8 groups yielded the following equation: $Y = 0.10 - 0.03\ X$ (X = extent of saliva spread, Y = heating rate, $r^2 = 0.98$). Thus, Figure 1 illustrates that increasing the extent (surface area covered) of saliva spread decreases heating rate resulting in a longer exposure time to reach a Tco of 42.6°C.

DISCUSSION

The administration of A to sedentary heat-stressed rats reduced thermal tolerance by suppressing the saliva secretion necessary for evaporative cooling. We have previously shown that this atropine-induced reduction in evaporative water loss rate in rats is quantitatively similar to that seen in man (7,8). The use of extent of saliva spread as an index of effective thermoregulation is analogous to "skin wettedness" (fraction of the human body surface wet with sweat) used in the quantitation of evaporative heat loss in man (13). When PH was administered with A, exposure time, % wt loss, and extent of saliva spread were all doubled while the heating rate was halved as compared to the group with A alone. Increasing the PH dose does not further improve results, because toxic effects are seen with higher doses of PH. The administration of D with A also improved thermal tolerance but not to the extent seen with PH. Higher doses of D have been shown by Vidal et al. to result in lowered Tco (14) and ataxia. While A+D+PH improved the thermal tolerance of atropinized rats when compared to A+PH, the difference was not significant.

Physostigmine administration stimulates salivation (as well as sweating) (3); thus, the current study indicates that the PH-treated rats initiate salivation before controls (Table II). However, total salivary water loss was similar in these 2 groups indicating that the heat stress itself was sufficiently intense to maximize salivary secretion. While atropine is a cholinergic receptor blocker, PH is a cholinergic agonist by virtue of its ability to bind acetylcholinesterase, thus increasing acetylcholine concentration at the receptor. It is possible that the agonistic effects of PH might not completely compensate for the antagonistic actions of A. PH administration has also been shown to induce a peripheral vasodilation which results in a decreased Tco in sedentary rats at ambient temperatures up to 26°C (1,15,16). A decreased Tco at the start of heat-stress should provide a thermoregulatory advantage (17,18) to the PH rats. However, PH's peripheral dilatory effect is blocked by A (15,16), and the ambient heat stress (41.5°C) combined with enhanced peripheral vasodilation could result in an actual heat gain rapidly negating any benefits of a lower starting Tco.

Diazepam has been shown to improve thermoregulatory ability by preventing febrile convulsions in children (19), by preventing handling induced hyperthermia in rats (14), and by decreasing the heating rate of rats running on a treadmill (9). D has also been shown to induce peripheral vasodilation which is blocked by A (20); as with PH, peripheral vasodilation would not be an advantage in a hot environment. D administration may have decreased the A-induced elevated heating rate in the A+D group by reducing metabolic rate and heat production. This hypothesis is supported by the work of Anholt et al. (21) indicating that peripheral benzodiazepine receptors may play a role in regulating metabolism, and preliminary work from our lab suggesting that administration of a dose of diazepam too low to be sedating still lowers oxygen consumption.

In the sedentary heat-stressed rat model, administration of diazepam and physostigmine to atropinized heat-stressed rats improves the thermal tolerance of atropinized rats but does not reduce heating rate to control levels. The improvement reflects primarily an increase in saliva available for evaporative cooling and perhaps a reduced metabolic rate. Additionally, the combination of A+D+PH restores PH-induced endurance decrements in the exercising rat model (9). Therefore, these 2 model systems can be used to examine the antimuscarinic and antinicotinic effects of the anticholinergics, and they are also useful in assessing the mutually antagonistic effects of the anticholinergics and the anticholinesterases.

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